Medical Progress

Sexual Differentiation: The Development Of Maleness and Femaleness

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ALL OF THE AVAILABLE EVIDENCE leads to the conclusion that in development the process of becoming a male involves a number of complicated steps which are unnecessary in order for femaleness to occur. Thus it appears that the basic primordial sexual differentiation of most mammalian species is female, and maleness is dependent upon a number of essential additive steps in the developmental process in order to produce those changes in reproductive physiology and behavior that characterize the male.

There are at least four major events which are needed in order for the developing embryo to become male. If any of these is altered significantly the tendency of the embryo is to continue in its development as a female or a genetic male with many female characteristics. Initially, in order for maleness to occur at all there must be present in the genes an X and a Y chromosome. The development of the female involves a double X chromosome. In humans 46 chromosomes are normally found; the major difference chromosomally between the male and the female is the presence of a Y chromosome in the male and the absence of a Y chromosome in the female. In humans who have been found to have 45 chromosomes it is due to the fact that they have only one X chromosome and lack a Y chromosome. Such XO persons are female. Other individuals have been found to have 47 chromosomes because they have two X chromosomes and a Y chromosome. Such XXY persons are males. The Y chromosome then determines maleness, normally in XY individuals, abnormally in XXY individuals. The absence of the Y chromosome determines females, normally in XX individuals, abnormally in XO individuals. This, then, is the first example of what we are calling an additive process; that is, the addition of a Y chromosome is necessary to initiate the very process of maleness.

The next critical event in becoming a male involves that of the formation of the male gonads, the testes. The mammalian sex organs, initially in development, appear as two genital ridges in the fetus. Each ridge has an inner mass of medullary tissue and an outer cortical mass. This indifferent gonad can go either in the direction of the formation of an ovary or the formation of the testes. If the medulla begins to reorganize itself the cortex atrophies and disappears, or conversely, if the cortex develops and the medulla retrogresses or disappears, then two different organs are formed. If the medulla develops, the resulting organ is a testis; if the cortex develops, then the resulting gonad is an ovary.

Recent information indicates that the formation of the testes precedes that of the formation of the ovaries in development. Although there is little information as to the mechanism whereby

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the indifferent genital ridge becomes either a testis or an ovary, it does appear that some active secretory process is necessary in order to cause the medullary tissue to develop. If this process does not happen, the tendency of the organism's indifferent genital ridge is to develop as an ovary. Once the testes are formed, however, there are still further vital steps that are necessary in order for the organism to become totally male. That is, the fetal testes must secrete a duct-organizing substance and then male hormones (androgens) in order for this embryo to become male. The secretion of a substance from the fetal male testes is absolutely essential for the anatomical differentiation of the male and for the functional differentiation of the male.

During intrauterine life the fetus is equipped with the primordia of both male and female genital ducts. The Müllerian ducts serve as an anlage of the uterus and fallopian tubes; whereas the Wolffian ducts have the potentiality of differentiating further into the epididymis, vas deferens, seminal vesicles and ejaculatory duct of the male. In humans, during the third fetal month, either the Müllerian or Wolffian ducts complete their own development while involution occurs simultaneously in the opposite structures. It has been clearly demonstrated by the eminent embryologist, Alfred Jost,[1] that secretions from the fetal testes play a decisive role in determining the direction of genital duct development. In the presence of functional testes the Müllerian structures involute while the Wolffian ducts complete their development; whereas in the absence of the testes the Wolffian ducts are reabsorbed and the Müllerian structures mature.

Female development is not contingent on the presence of an ovary, since equally good development of the uterus and tubes will take place if no ovary is present. The influence of the fetal testes on duct development is unilateral since early removal of one testis leads to Müllerian development on that side while the male duct normally develops on the side on which the testis remains intact. The systemic injection of androgen to an early embryo fails to duplicate the action of the fetal testes. When androgen is applied locally under conditions of extremely high dosage the Wolffian ducts exhibit signs of stimulation, but no inhibitory effect on Müllerian elements has been observed. These data have led to the belief that the fetal testes secrete a duct-organizing substance which is distinct from androgen.

However, the presence of the normal male anatomical structure still does not insure that the developing embryo will become functionally male. What do we mean by the concept of functionally male? If we were to examine in detail some of the major differences between male and female sexuality we would note that there are sex dimorphisms in many aspects of reproductive physiology, stress physiology, metabolic processes, and behavior. In particular, with regard to reproductive physiology and the behavioral aspects of maleness and femaleness, it does appear that one of the principal actions of androgen during development is to organize the immature central nervous system into that of the male. Once again we are talking about an active process; that is, the presence of androgen during development acts upon the brain to program, in effect, patterns of maleness.[2,3] The absence of androgen permits the ongoing process of femaleness to pursue its natural course. The evidence to support this theory is now abundant.

Let us begin by examining one of the principal distinctions between males and females with regard to reproductive physiology. In most species of mammals the female has a cyclic pattern of ovulation. The human female ovulates about every 28 days, the guinea pig about every 15, and the rat every 4 to 5 days. In cyclic fashion the anterior pituitary delivers to the ovary follicle-stimulating hormone (FSH) which promotes the growth of the Graafian follicle which produces estrogen and also houses the ova to be released at the times of ovulation. The anterior pituitary also releases luteinizing hormone (LH) which induces the formation of the corpora lutea and triggers ovulation. The formation of corpora lutea is clear evidence that ovulation has occurred. There is a continuing ongoing feedback system which is only interrupted in the normal cycling female by the onset of pregnancy. The male, in contrast, shows no such cyclicity. Its testes continually receive from the pituitary luteinizing hormone, but this luteinizing hormone in the male causes the development of the interstitial cells of the testes, those cells which are predominantly responsible for testosterone production. Thus, the pattern of hormone production from the pituitary, in order

to maintain the process of ovulation in the female, is indeed a cyclic one. The process of production of hormones from the male is for the most part noncyclic.

It has been demonstrated that the pituitary is in itself not sexually differentiated,[4] so that if a female pituitary is transplanted into a male, normal male functions will be maintained. Conversely, if the male pituitary is transplanted into a normal female, complete female function will also be maintained. The implications of these studies are that pituitary regulation comes not from the pituitary itself but from some other controlling mechanism. All of the available evidence indicates that the controlling mechanisms are somewhere in the central nervous system. The clearest demonstration of the role of the testes in modulating central nervous system control of reproduction comes from studies in which newborn rats have been deprived of their testes.

It has been dramatically demonstrated, most recently by Professor Geoffrey Harris[2] that when these neonatally castrated males are allowed to grow, their pattern of pituitary release of those hormones which regulate gonadal function (FSH and LH) is cyclic and indistinguishable from that of a normal female. Thus, if an ovary is transplanted into the adult animal who has been castrated as a newborn, this ovary shows full cyclic ovulation. Thus, we have a case where a genetic male with XY chromosomes, a morphological male showing all of the sexual differentiation of anatomical structures, still is responding, in the central nervous system, as a female. It should be noted that if, following castration, the male is given a single injection of testosterone, the ovulation that is seen in adulthood no longer occurs. Furthermore, if the newborn female is given a single injection of testosterone shortly after birth, she is also acyclic and incapable of maintaining normal patterns of ovulation.[5]

These studies provide one line of evidence which indicates that the maleness is dependent upon having a male central nervous system and that in order to have a male central nervous system the fetal and neonatal testes must secrete androgen which presumably acts upon the brain to differentiate it into that of the male. There are definite critical periods in development for these events to occur; thus, in the rat if the newborn male is castrated within 24 hours after birth the central nervous system will continue to be female. If, however, this period exceeds approximately 72 hours, then the process is now irreversible and the male central nervous system has been permanently established.

The hypothesis of sexual differentiation of the brain is further substantiated when one closely examines the influence of the presence or absence of testosterone in both the newborn male and female rat on adult sexual behavior. In most mammalian species the hormones emanating from the ovary and the testes have profound control of sexual behavior. In normal circumstances the female rat becomes sexually receptive during a period in the estrous cycle when there exists the appropriate hormonal balance between the ovarian hormones, estrogen and progesterone, that results in ovulation.[6] If the female is deprived of the appropriate circulating hormones by ovariectomy, sexual receptivity is immediately abolished. However, when the appropriate hormones are replaced, either in the form of chronic high doses of estrogen, or small doses of estrogen followed by progesterone, sexual behavior appears within a very short time following progesterone administration. Sexual behavior of the male rat involves a much more complex pattern of mounts with intromissions and ejaculation. In contrast to the cyclic pattern of receptivity exhibited by the female, the male is acyclic in his sexual behavior and will under normal circumstances copulate as long as there is an appropriate stimulus object.

The biological adaptiveness of these two different patterns is readily apparent; thus, for the female of most mammalian species, sexual receptivity is consistent with ovulation, so that almost every sexual contact would result in pregnancy. However, if the male also had a cyclic pattern of sexual activity, then the conditions under which pregnancy would occur would at least be infinitely more complex. Again, in contrast to the female, when the male is castrated there ensues a period of time during which the male is sexually active, even in the absence of circulating hormones. Eventually, however, the male will cease normal sexual activity and, following replacement with testosterone, will resume behavior that is indistinguishable from the normal intact male. However, no amount of estrogen and progesterone has yet proved capable of reliably eliciting, in an adult castrate male, patterns of sexual behavior that are typical of the normal female.

The evidence regarding normal patterns of sexual behavior and their dependence upon circulating hormones is consistent with the hypothesis that there are differences between the male and female brain with regard to patterns of hormone secretion and behavior. Thus, female sexual receptivity is easily elicited with the appropriate regime of estrogen and progesterone replacements following removal of the ovary. In the male these behaviors appear to be completely suppressed and cannot be elicited with doses of estrogen and progesterone that are a thousand-fold higher than those required in the female. Therefore, one of the primary aspects of sexual differentiation in the rat appears to be the suppression of the capacity in the normal male to respond to estrogen and progesterone.

Although there is a firm underlying assumption that behavior reflects in some ways the action of the central nervous system, there is clear evidence that sex hormones can act directly on the brain. Implants of synthetic estrogen (stilbestrol) in one area of the brain, the hypothalamus, of female cats evoke female sexual behavior, although the cats do not show the usual physiological signs of estrus.[7] In similar experiments implants of testosterone in the brains of castrated male rats also elicited male sexual behavior, although again there was no sign of the effect of this testosterone on the anatomical structures of the male reproductive system.[8]

The influence of androgen during development on sexual behavior patterns is demonstrated by the now two classic approaches to the problem: one is the administration of testosterone to an organism which normally would not have testosterone—that is, the female—and the other is the removal of the testosterone-producing organs, the testes, during a critical period in the development of the male. In the case of the female, a single injection of an appropriate dose of testosterone[9] is capable of abolishing female patterns of sexual receptivity. This female not only fails to show any signs of sexual receptivity under normal circumstances but also when the ovary is removed sexual behavior cannot be elicited by the appropriate replacement of estrogen and progesterone which normally would elicit complete sexual receptivity in non-testosterone treated females with ovaries removed. Further, these females treated neonatally with testosterone will show some increase in male patterns of sexual behavior following injections of androgen in adulthood.[5] Conversely, although it is extremely difficult, if not impossible, to elicit female receptivity in a male that has been castrated as an adult, when the testes are removed within 24 hours after birth sexual responses elicited by estrogen and progesterone in adulthood are completely indistinguishable from those of a normal female.[10] Not only are they indistinguishable to the human observer but normal adult males will respond to these estrogen and progesterone treated neonatal castrated males as if they were females in heat. Once again, a single injection of testosterone given shortly after castration to the newborn animal will completely reverse all of these effects.

We have thus far assumed that the function of gonadal hormones in infancy is to organize the central nervous system with regard to neuroendocrine control of behavior. Although we have focused primarily on reproductive behavior, numerous reports in the literature have indicated that there are sex differences in nonsexual behavior. If indeed we are to make a convincing argument that the effects of androgen are to influence the central nervous system, then it seems reasonable to assume that other patterns of sex differences would also be influenced by these same hormones. There are now well-reported differences[2] in activity patterns between males and females. Activity patterns of the female closely parallel estrous cycle activity and during the estrous phase of the cycle females show high peaks of activity. In contrast, the male shows no apparent activity cycle and over-all activity levels are much lower. These female activity cycles can be mimicked in the neonatal castrated male by an ovarian transplant in adulthood. Thus, before the transplantation of the ovary the male which has been castrated as a newborn, shows a low level of random activity. However, the appearance of the corpora lutea in the transplanted ovary marks the onset of female activity cycles which are again indistinguishable from those of normal females. During this period the neonatally castrated rat with the transplanted ovary also becomes sexually receptive in cycles. Sex dimorphisms have also been noted in other patterns of behavior, including emotional responses to novel situations, and aggressive behavior.

Recently we have investigated[11] the effects of androgen on experimentally induced aggressive

behavior. Males and females differ markedly in the amount of aggressive behavior elicited by exposure to electric shock, with males fighting significantly more than females. Further, if males are castrated at weaning, their aggressive behavior is reduced but not quite to the level seen in normal females. However, when they receive replacement treatment with testosterone these males show significant increases in aggressive behavior equivalent to that observed in the normal intact male. The female, however, shows no increase in aggressive behavior following testosterone treatment. In male rats castrated as newborns, aggressive behavior is suppressed and supramaximal doses of androgen given to the adult organism do not increase aggressive behavior as seen in castrated weanling rats. Thus again we have an example of the maintenance of feminine patterns of behavior when the newborn male is castrated. Further, there appears in this experiment that property of the central nervous system which has been observed throughout many of the experiments discussed thus far, namely that the female brain is differentially responsive to androgen and that many behaviors which are elicited by androgen in the normal males are generally incapable of appearing on androgen stimulation in the normal female or its equivalent, the male that is castrated during the critical period in development.

Examination of developmental patterns of the testes indicates that during the late prenatal periods and in the rat for a brief period of time postnatally, the fetal and neonatal testes exhibit a high degree of endocrine activity. There is active production of testosterone from the testes. However, after this brief period of activity the testes become very quiescent and there is very little androgen production until just before puberty. One can infer that this period of high androgen activity during a critical period in development is essential for the sexual differentiation of the brain.

For obvious reasons, the research we have discussed thus far has been accomplished mainly in laboratory animals. The closest human analogue which demonstrates the influence of testosterone during development on subsequent sexuality is the syndrome of testicular feminization. Clinically, the internal ducts are predominantly male. The external genitalia, however, resemble those of the female, although the vagina is shallow and ends blindly in a pouch. At adolescence, female secondary sex characteristics develop – notably well-developed breasts and rounding of the body contours. Etiologically this disorder can be attributed to a peculiar process by which the target tissues become androgen resistant. Thus, although the testes produce the appropriate hormones, apparently the tissues remain insensitive to this hormone and consequently produce the syndrome of overt feminization. The clinical literature is abundant with numerous instances of gender role reversals as a consequence of pathological conditions which result in sexual ambiguities, in particular, penis-like structures in female offspring. However, it has been clearly demonstrated by Money and co-workers[12] that to a very large extent the gender role assigned to the individual who is born with distorted external genitalia is dependent on the way in which the individual is reared. Thus if the individual with such sexual ambiguity is treated as a male, it will generally assume a male gender role, although it may indeed have ovaries. Conversely, if the individual is treated during development as a female, it will thus continue female, although again the internal genitalia and chromosomal patterns may be those of a male. Thus, at least as far as the human is concerned, there is an additional process in development which involves the establishment of gender role as a function of learning.

What we have tried to demonstrate in this paper is that for maleness to occur there are several unique active processes which must take place with exquisite timing during development. Perhaps of more importance is the influence that hormones have on the developing central nervous system to organize and establish patterns of physiology and behavior that will determine the organism's life history. All the experimental evidence presented here supports the view that alterations in hormonal status in the newborn animal have profound and permanent effects on the animal's subsequent biological functioning.

Although we have dealt specifically with only the gonadal hormones, there is evidence[13,14] indicating that both changes in thyroid and adrenal function can also permanently affect the developing nervous system. Although we do not as yet have any evidence as to the mechanisms whereby these hormones act upon the central nervous system, we believe that this is indeed one of the more

exciting, newer concepts to emerge from developmental biology and if nothing else, indicates that the developing organism is extremely sensitive to hormones and certainly suggests a judicious use of hormones during those critical periods in the development of the organism which may be most profoundly affected by hormonal changes in the environment.

REFERENCES

1. Jost A: Embryonic sexual differentiation, *In* Jones HW, Scott WW (Eds): Hermaphroditism, Genital Anomalies and Related Endocrine Disorders. Baltimore, Williams & Wilkins Co, 1958, pp 15-45

2. Harris GW: Sex hormones, brain development and brain function. Endocrinology 75:627-648, 1964

3. Young WC, Goy RW, Phoenix CH: Hormones and sexual behavior. Broad relationships exist between the gonadal hormones and behavior. Science 143:212-218, 1964

4. Harris GW, Jacobsohn D: Functional grafts of the anterior pituitary gland. Proc Roy Soc London (B) 139:263-276, 1951/1952

5. Harris GW, Levine S: Sexual differentiation of the brain and its experimental control. J Physiol 181:379-400, 1965

6. Young WC: The hormones and mating behavior, *In* Young WC (Ed): Sex and Internal Secretions. Baltimore, Williams & Wilkins Co, 1961, pp 1173-1239

7. Harris GW, Michael RP: The activation of sexual behaviour by hypothalamic implants of oestrogen. J. Physiol 171:275-301, 1964

8. Davidson JM: Activation of the male rat's sexual behavior by intracerebral implantation of androgen. Endocrinology 79:783-794, 1966

9. Mullins RF Jr, Levine S: Hormonal determinants during infancy of adult sexual behavior in the female rat. Physiol Behav 3:333-338, 1968

10. Mullins RF Jr, Levine S: Hormonal determinants during infancy of adult sexual behavior in the male rat. Physiol Behav 3:339-343, 1968

11. Conner RL, Levine S: Hormonal influences on aggressive behavior, *In* Garattini S, Sigg EB (Eds): Aggressive Behaviour. Amsterdam, Excerpta Medica, 1969, pp 150-163

12. Hampson JL: Determinants of psychosexual orientation, *In* Beach FA (Ed): Sex and Behavior. New York, John Wiley & Sons, 1965, pp 108-132

13. Levine S, Treiman LJ: Role of hormones in programming the central nervous system, *In* Wolstenholme GEW, O'Connor M (Eds): Ciba Foundation Symposium on Foetal Autonomy. London, J & A Churchill Ltd, 1969, pp 271-281

14. Levine S, Mullins RF Jr: Hormonal influences on brain organization in infant rats. Science 152: 1585-1592, 1966

LID RETRACTION AS A SIGNAL OF CHANGE IN THYROID FUNCTION

"One of the things that often points to changes in thyroid function is upper lid retraction. At least in the patients we have seen, this problem occurs as frequently without exophthalmos as it does with it. It may go unnoticed for a number of years. Often the lid will gradually creep up over a period of time and then stop. The difficulty that comes with it is due to exposure of the lower portion of the cornea when the patient is asleep. These individuals do not completely close their eyes; the cornea rolls down slightly and one gets stippling along the lower portion of the limbus. This stippling often leads to complaints by the patients . . . of a foreign body sensation. It's not very evident by routine slit-lamp examination. Usually you have to stain the cornea in order to see it. If you're going to check for inability to close the lids, ask the patient to close his eyes as if he were sleeping because virtually all of these patients can get their lids against each other if they clamp down real hard. You really want to know what's happening when they're relaxed and the lids are just lying against the globe."

—James E. Miller, M.D., Albany, N.Y.
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